really be considered as true verbs. Then we must try to clear them of all the artificial additions of the priests, and to find out the real Indian verb and all its possible forms, tenses and moods, a task by no means so easy as it would seem from a superficial examination. In a subsequent publication, I propose to enter more fully upon this theme.

On the Embryology of Limulus polyphemus. III. *By A. S. Packard.*

(*Read before the American Philosophical Society, January 16, 1885.*)

The stage under examination is that represented on figs. 12 and 13, 14 and 15 (Plates iii and iv), of my essay on the development of Limulus, Memoirs Boston Society Natural History, 1872. At this stage the oval blas-todermic disc, with the six pairs of the cephalic appendages, is distinctly formed ; the mouth is seen in a position in front of the first pair of append-ages, and from it the primitive streak passes back to the posterior margin of the blastodermic disc or "ventral plate." The abdomen is separated from the head by a curved groove, as seen in fig. 12, of my memoir.

I should here remark that the eggs were not fresh, but selected from a number kindly collected for me in 1871, by Rev. Samuel Lockwood, and since then preserved in alcohol, which had been renewed several times, my studies on the embryology of this animal having been interrupted from year to year, in hopes of obtaining fresh eggs, and for want of good thin sections of those I already had. I finally applied to my friend Dr. C. O. Whitman, whose great experience in making delicate sections was kindly placed at my disposal ; the sections examined were actually made by Mrs. Whitman, under the direction of her husband. The period ex-amined is an interesting one, as while the cephalic appendages were well-developed, the abdominal appendages were not as yet indicated, nor the post-oral nervous ganglia.

The first point, which at once excited my attention, was the nature of the embryonic membrane which I had previously regarded as the homologue of the amnion, and afterwards as the serous membrane of insects, but which Mr. J. S. Kingsley* has found to be secreted from the blastoderm. That he was correct, and that I was in error in regarding it as truly cellular, was at once seen to be evident. A thin section (fig. 1 and 5), shows that the membrane is very thick, structureless, the cellular appearance being con-fined to the external surface. This membrane is evidently secreted by the blastoderm ; the irregular cell-like markings (see my second memoir, 1880, Pl. iii, figs. 14, 14a, 14c, 14d), are, so to speak, casts of the blastoderm cells, which with the marks of even their nuclei are impressed upon the

* The Development of Limulus, Science Record, ii, pp. 249-251, Sept., 1884.

membrane during the early stage in its formation ; after a while new matter is added to the interior which is structureless, so that the cellular appearance is only superficial. In my comparison of this membrane with the serous membrane I certainly exaggerated its resemblance to the *serosa* of insects, as the latter is a much more delicate membrane, and with a characteristic appearance in Crustacea, the scorpion, myriopods and hexapods. The membrane in question appears to have its homologue, however, in the embryonic membrane of Apus, which we have described in a foot-note on p. 161, of our first memoir. It thus appears that this supposed point of resemblance in Limulus to the Tracheata is removed.

A longitudinal section of the embryo of Limulus is represented by fig. 2. The section passes through the blastodermic disc (ventral plate) and the indications of the appendages, on one side of the median line of the body. The epiblast entirely surrounds the yolk, forming a thin layer with nuclei, the cell walls not being distinct, while the nucleolus consists of a number of granules. The nuclei are two-deep only on the cephalic portion of the embryo. The blastodermic disc does not extend quite half-way around the egg. The six pairs of appendages are well-developed, increasing in size from the first to the last pair. The mesoblast is now well developed ; the nuclei well marked, but the cellular walls more or less effaced. The mesoblastic arthromeres are now well indicated. The somatic cavities are well marked in each appendage ; the somatopleure is from one to three cells deep ; the splanchnopleure is formed usually of two layers of cells, and is more or less continuous at the ends of the somatic cavities with the somatopleure. The relations of these divisions of the mesoblast, which are destined to form the muscles of the limbs and the ventral aspects of the body, are represented in fig. 3.

The mesoderm, as seen in fig. 3, is now differentiated into three sub-layers : 1, the somatopleure ; 2, the splanchnopleure, and 3, a sublayer from which probably arises, in part at least, the connective tissue so remarkably developed in the head of Limulus ; in its thickest portion at this stage this innermost layer consists of about eight series of cells, which are more loosely arranged than in the sublayers next to the epiblast.

The yolk granules are minute, the largest granules not more than twice as large as the nuclei of the mesoderm. The hypoblast cells are by far the largest cells in the embryo, and at once attract attention by reason of their size, and their deep color when stained ; the nucleus and nucleolus are well marked. At this stage no hypoblast cells could be detected in the yolk, nor any protoplasmic network connecting them. Those present formed a dorsal row ranged next to the thin epiblast over about one-quarter of the periphery of the ovum. In an earlier stage, however, the yolk granules are contained in distinct polygonal cells, forming a network extending through the yolk.

The abdomen has not yet undergone segmentation ; the incipient steps are represented in fig. 2, where there appear to be arising five mesoblastic segments (1, 2, 3, 4, 5). Between the first and second mesoblastic mass

is a narrow cavity which sends a branch forward to the base of the abdomen, and a second obliquely downward and inward ; at 2 and 3 in fig. 2, there are narrow cavities or splits (somatic cavities ?) which communicate with a longitudinal internal opening, which extends in a direction parallel to the under (now outer) surface of the abdomen. In this respect the embryo of Limulus is very different from that of the scorpion and spiders (see especially Balfour's figs. 5, 6, Pl. xix and fig. 15, Pl. xx), where the abdominal segments, with their appendages and somatic cavities are formed contemporaneously with those of the cephalothorax.   The innermost mesodermic cells are now arranged in long cords, destined to form the ventral adductor muscles of the abdomen.

The mode of formation of the head and its shape at this time presents important differences from that of tracheate embryos. The procephalic lobes are not developed ; the preoral portions of the head, *i. e.*, that part in front of the first pair of limbs is very small, short and narrow, merely forming the end of the oral blastodermic disc, seen in my earlier published figures. The structure of the preoral portion of the head (*procephalum* as we may term it), is seen in longitudinal section in fig. 3, *pc*, to apparently consist merely of an extension of the postoral part of the head ; with apparently one or two splits in the mesoderm (ms^1, ms^2), the nature of which I do not understand ; undoubtedly farther sections and comparisons will throw light upon it. There is no involution of the epiblast, and the section passes one side of the mouth, a good section of which I have not yet seen.

The first nervous ganglion is seen at fig. 5, to result (as also first shown by Kingsley) in an ingrowth of the epiblast (*inv. c*) ; carrying into the interior a mass of epiblastic nuclei, which envelop the myeloid substance (*my*), which, as in older embryos, remains unstained by the carmine.

The mesoblastic nuclei stop at a large cell (*c*), beyond which are long incipient loose muscle-cells, with a few scattered nuclei. The procephalum terminates abruptly, forming, as seen in our earlier figures already referred to, the end of the blastodermic disc.

The absence of the procephalic lobes in the embryo Limulus of this stage seems to us to be a very significant fact, and to point to the early divergence of the Palæocarida from the stem leading up to the Tracheata, and especially the Arachnida. Metschnikoff's researches on Scorpio, with those of Claparède, and of Balfour on the spiders, and those of Sograff on the myriopods, show that this is a fundamental and early attained feature in these types. Their absence in Limulus shows how little its embryo has in common with tracheate embryos. At the same time the general mode of formation of the blastodermic disc (ventral plate) of Limulus is much like that of the spider, as seen in the mode of origin of the mesoblastic segments, and the probable orgin of the hypoblastic cells. There is a superficial resemblance between the embryo of Limulus and of the spider, as may be seen by a comparison of our fig. 2, and Balfour's fig. 15. Without much doubt the Tracheata and Palæocarida, as well as Crustacea

Neocarida, branched off from a common ancestor, but the more important morphological points show that the terrestrial, air-breathing tracheates were a much later branch of the Arthropod tree than the marine branchiate Palæocarida and genuine Crustacea. Probably the Palæocarida (Limulus, Merostomata and Trilobita) were the earliest arthropods to appear; after them arose the Crustacea, perhaps at nearly the same time the Arachnida, and finally the Myriopoda and the winged insects. Without much doubt, the earliest branchiate forms were our Protocyclus,* the ancestor of the Palæocarida; and a Protonauplius form, the forerunner of the Crustacea; these were marine, perhaps branchiate organisms, with a few pairs of simple oar-like swimming appendages either around or just behind the mouth, and which were free swimming or creeping forms; the Protocyclus was, perhaps a solid oval creeping animal living at the bottom on mud or sand. The branchiæ probably became first developed on the limbs of the free-swimming Protonauplii, as they needed, owing to their great rapidity of movement, the means of rapid aëration of the blood; while in the heavily-moulded less oxygen-consuming Protocyclus, the evolution of gills was somewhat postponed. The steps from Protocyclus to Agnostus was not a very long one. The oldest arthropods, notwithstanding the recent discovery of a Silurian scorpion, were trilobites.

The following conclusions are drawn from a study of the stage of Limulus here figured.

The fact that the embryo Limulus has at first no abdominal appendages (uropoda), whereas there are temporary abdominal appendages in the tracheates, shows that Limulus in this important respect has little in common with the Arachnida, Myriopoda or Hexapoda. On the other hand in the embryo Crustacea the cephalic limbs are first indicated; the nauplian limbs, as well as the zoëan appendages being cephalic; the uropods not appearing until after the Crustacea leave the egg.

These facts indicate that Limulus probably descended from a type in which there were cephalic appendages only, and no abdominal appendages. The absence of a serous membrane, of an amnion, and of procephalic lobes, of temporary embryonic abdominal appendages (at the stage above described); also of protozonites (seen in the early embryo of the scorpion and spider) tend to prove that the embryo of Limulus has little in common with that of Tracheata.

On the other hand the earlier stages in the embryology of Limulus resemble those of Crustacea in the absence of the procephalic lobes; in the primitive development of cephalic appendages alone; the comparatively early appearance of the branchiæ of Limulus in the stage succeeding that figured in this essay, shows that the Limulus probably never had any genetic connection with a tracheate arthropod.

On the other hand, the tracheate features of mesoblastic somites are also seen in the worms, in Peripatus and in Annelida.

* See Development of Limulus, 1872, p.

It appears that the embryology of Limulus is scarcely more like that of tracheates than Crustacea; it is a very primitive type standing nearer the branchiate arthropods than the tracheate, but on the whole should be regarded as a generalized or a composite form, which with its fossil allies, the Eurypterida and Trilobitæ, form a class by themselves with a superficial resemblance to the Arachnida.

It seems to us that the above-mentioned characters, which separate the early embryo of Limulus from the tracheates, are as important, if not much more so, than those of the absence at first of an archenteric cavity or differences in the mode of origin of the mesoblast, noted by Mr. Kingsley in his brief paper on the development of Limulus. In these general, primitive embryonic characters Limulus appears to be as nearly allied to the annelids as to the tracheates; and too much dependence should not, it seems to us, be placed upon them in seeking to establish the true relations of the Palæocarida among the arthropods. In the higher worms the two longitudinal mesoblastic bands split into somatic and splanchnic layers (Kowalevsky). In Mysis Metschnikoff states that the mesoblast becomes broken up into distinct somites (Balfour's Embryology i, 436). If so, then this character is not one of much importance to separate Limulus from the Crustacea. The ultimate origin of Limulus from the same stock as that which gave rise to the modern annelids seems not improbable.

EXPLANATION OF THE PLATE.

Fig. 1.—Blastodermic cuticle (*bl. cut*) lying upon the epiblast (*ep*). The nuclei scattered through the latter; the nucleolus, in these as well as the mesoblast cells, consisting of a number of granules. $\times \frac{1}{5}$ A.

Fig. 2.—Longitudinal section through an embryo before the appearance of the abdominal appendages, but after the rupture of the chorion; the section passes through the six cephalic appendages (i-vi), showing the somatic cavities (*ms*), the splanchnopleure (*sp*), and somatopleure (*so*), 1–5, the indications of the five primitive uromeres; *hy*, hypo or ectoblast $\times \frac{1}{5}$ A.

Fig. 2a.—Showing the relations of the hypoblastic cells (*hy*) to the epiblast (*ep*) in the dorsal region of the embryo.

Fig. 3.—Longitudinal section of the head and the first three appendages; ms^1, ms^2, first and second somatic cavities in the preoral region of the head. This figure also shows the relations of the splanchnopleure and somatopleure to the epiblast. *c*, large distinct cell in preoral region. $\times \frac{1}{5}$ A.

Fig. 4.—Transverse section through the head, including the appendages. $\frac{1}{5} \times$ A.

Fig. 5.—Transverse section through the head, showing the invagination, and thickening of the epiblast to form the brain; *my*, myeloid substance of the ganglion. $\times \frac{1}{5}$ A.

All the longitudinal sections are from the same egg, and the transverse sections from another. The figures were all drawn by the author with the camera.